WEST Search History

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DATE: Tuesday, July 13, 2004

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI; PLUR=YES; OP=ADJ	
☐	L1	6270966.pn. and polymerase	2
☐	L2	L1 and promoter	0
☐	L3	L1 and (adapter or adaptor or linker)	2
☐	L4	in vitro transcription	1691
☐	L5	L4 (adapter or adaptor or linker)	0
☐	L6	L4 and (adapter or adaptor or linker)	959
☐	L7	L6 and (RNA polymerase promoter)	237
☐	L8	L7 and restriction endonuclease	140
☐	L9	L8 and (differiental display or gene expression)	129
☐	L10	(first strand synthesis or synthesis) and l9	129
☐	L11	L10 and (fluorescen$-label$ primer or fluorescen$-label$ oligo-dT)	1
☐	L12	L10 and oligo-dT	18
☐	L13	(first strand synthesis) and l9	22
☐	L14	(NASBA or nucleic acid sequence based amplification)	3219
☐	L15	L14 and (adapter or adaptor or linker)	1900
☐	L16	L14 and ((adaptor or adapter or linker) same polymerase same promoter)	63

END OF SEARCH HISTORY

describe construction of a randomized siRNA gene library under control of a U6 snRNA promoter, construction of an siRNA expression vector with a tetracycline-inducible promoter, and down-regulation of firefly luciferase in a breast cancer cell line (MCF7-luc) by plasmid pLPR-U6-lucB-siRNAh. Another example describes use of a hairpin siRNA gene library to enrich for siRNAs that down-regulate surface CD4 expression in the human T cell line, Molts-4.

L2 ANSWER 2 OF 19 MEDLINE on STN DUPLICATE 1

ACCESSION NUMBER: 2004081263 MEDLINE
DOCUMENT NUMBER: PubMed ID: 14970461
TITLE: Small amplified RNA-SAGE.
AUTHOR: Vilain Catheline; Vassart Gilbert
CORPORATE SOURCE: Institute of Interdisciplinary Research (IRIBHM), Univeriste Libre de Bruxelles, Brussels, Belgium.
SOURCE: Methods in molecular biology (Clifton, N.J.), (2004) 258 135-52.
Journal code: 9214969. ISSN: 1064-3745.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200405
ENTRY DATE: Entered STN: 20040219
Last Updated on STN: 20040514
Entered Medline: 20040513

AB Serial analysis of gene expression (SAGE) is a powerful genome-wide analytic tool to determine expression profiles. Since its description in 1995 by Victor Velculescu et al., SAGE has been widely used. Recently, the efficiency of the method has been emphasized as a means to identify novel transcripts or genes that are difficult to identify by conventional methods. SAGE is based on the principle that a 10-base pair (bp) cDNA fragment contains sufficient information to unambiguously identify a transcript, provided it is isolated from a defined position within this transcript. Concatenation of these sequence tags allows serial analysis of transcripts by sequencing multiple tags within a single clone. Extraction of sequence data by computer programs provides a list of sequence tags that reflect both qualitatively and quantitatively the gene expression profile. Several modifications to the initial protocol allowed to start from 1 microg total RNA (or 10(5) cells). In order to reduce the amount of input RNA, protocols including extra polymerase chain reaction (PCR) steps were designed. Linear amplification of the mRNA targets might have advantage over PCR by minimizing biases introduced by the amplification step; therefore we devised a SAGE protocol in which a loop of linear amplification of RNA has been included. Our approach, named "small amplified RNA-SAGE" (SAR-SAGE) included a T7 RNA **polymerase promoter** within an **adapter** derived from the standard SAGE linker. This allowed transcription of cDNA segments, extending from the last NlaIII site of transcripts to the polyA tail; these small amplified RNAs then serve as template in a classical (micro)SAGE procedure. As the cDNAs are immobilized on oligo(dT) magnetic beads, several rounds of transcription can be performed in succession with the same cDNA preparation, with the potential to increase further the yield in a linear way. Except for the transcription step itself, the present procedure does not introduce any extra enzymatic reaction in the classical SAGE protocol, it is expected to keep the representation biases associated with amplification as low as possible.

L2 ANSWER 3 OF 19 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN DUPLICATE 2

ACCESSION NUMBER: 2003-00491 BIOTECHDS
TITLE: Novel adaptor sequences for rapid joining with a target nucleic acid sequence, comprise topoisomerase recognition/cleavage sequence and a functional group or

encoded functionality;
DNA adaptor for polymerase chain reaction and target genome or DNA detection

AUTHOR: YAROVINSKY T
PATENT ASSIGNEE: YAROVINSKY T
PATENT INFO: US 2002068290 6 Jun 2002
APPLICATION INFO: US 2001-871607 31 May 2001
PRIORITY INFO: US 2001-871607 31 May 2001; US 2000-208662 31 May 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-582473 [62]
AN 2003-00491 BIOTECHDS
AB DERWENT ABSTRACT:

NOVELTY - An adaptor (I) comprising a first nucleic acid with 5' and 3' ends and scissile strand topoisomerase I cleavage (SSTC) motif with 5' motif sequence contiguous with 3' motif terminal nucleotide which is contiguous with palindromic sequence of 2-10 nucleotides contiguous with 3' end nucleotide that is complementary to 3' motif terminal nucleotide of SSTC motif, is new.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a nucleic acid (II) with a 5' and 3' end comprising a first functional nucleotide sequence and a SSTC motif sequence located 3' to the first functional nucleotide sequence and provides SSTC site that is not more than 10 bases from the 3' end of the nucleic acid.

WIDER DISCLOSURE - Disclosed are: (A) creating topoisomerase I-activated adaptor sequences; (B) target nucleic acids and its homologs or portions; (C) isolated nucleic acids comprising a sequence encoding target polypeptides; (D) variants and/or equivalents of the above nucleic acids; (E) nucleic acid molecules for use as probes or primers or antisense molecules; and (F) a nucleic acid which hybridizes under stringent conditions to a specified nucleic acid.

BIOTECHNOLOGY - Preferred Adaptor: (I) further comprises a second nucleic acid with a 5' end sequence that is complementary to the 5' motif sequence of SSTC motif. The 3' motif terminal nucleotide of SSTC motif is T and the 5' motif sequence of SSTC motif is CCCT or TCCT. The first nucleic acid further comprises a restriction endonuclease site located 5' to SSTC motif, and a 5' end sequence that is complementary to the 5'-overhang of a restriction endonuclease site. The restriction endonuclease site is BamHI, BglII, ClaI, DdeI, EaeI, EagI, EcoRI, HindIII, KasI, MboI, MluI, NcoI, NdeI, NheI, NotI, PaeR7I, SalI, Sau3A, SpeI, StyI, XbaI, XhoI or XmaI. The first nucleic acid further comprises first functional nucleotide sequence such as prokaryotic, eukaryotic or viral promoter sequence, mutational sequence, polypeptide tag encoding sequence, a nucleic acid tag sequence, terminator sequence, a fusible protein encoding sequence, a radioactively labeled nucleotide sequence, a chemically labeled nucleotide sequence or an intronic sequence. The first nucleic acid further comprises a functional nucleotide sequence that is 5' to SSTC motif. The functional nucleotide sequence is a phage promoter such as SP6, T3 or T7 promoter. Preferred Nucleic Acid: In (II), SSTC motif sequence is CCCTT or TCCTT.

USE - (I) is useful for joining an adaptor sequence to a target nucleic acid sequence, by providing (I), and a target nucleic acid with a base 3' overhang nucleotide that is complementary to the 3' motif terminal nucleotide of SSTC motif, and incubating (I) with the target nucleic acid in the presence of topoisomerase I activity. The method also involves amplifying the joined product by polymerase chain reaction (PCR) utilizing a first primer specific to (I) and a second primer specific to the target nucleic acid sequence. The target nucleic acid is generated by PCR of a target genomic or complementary deoxyribonucleic acid (cDNA) sequence with a 5' sense strand primer and a 3' anti-sense strand primer. The method further involves preparing at least two separate amplification reactions from the joined product comprising first amplification reaction with 3' antisense strand primer and a first adaptor primer, and second amplification reactions with 5' sense strand primer and a second adaptor

primer, where the adaptor primers comprise sequences in the nucleic acids of the adaptor. The method further involves isolating the product of either the first or second amplification reaction, and contacting the amplification product with a ribonucleic acid (RNA) polymerase activity which recognizes the promoter sequence (all claimed).

EXAMPLE - Preparation of universal adaptors for incorporation of a T7 RNA **polymerase promoter** was performed as follows. The **adaptor** preparation started by hybridization of two synthetic oligonucleotides such as T7TOPO (i) and ASTOPO (ii), respectively. DNA hybrids were created by combining equimolar amounts of T7TOPO and ASTOPO oligonucleotides at 65degreesC followed by slow cooling. Hybridization formed a stable complex of oligonucleotides with two recognition sites within the DNA duplex. This complex was stored in 50% glycerol at -20degreesC. Adaptor activation was performed by incubation of 8 pmol hybrid DNA with 5 units of vaccinia virus topoisomerase 1. Polymerase chain reaction (PCR) products generated from genomic DNA and single-stranded cDNA were generated as target nucleic acids for incorporation of T7 promoter sequences using the topoisomerase activated adaptors. Two oligonucleotides, corresponding to sense and antisense sequences of the human PRL-1 gene were used to amplify a 483 bp fragment of the gene from human genomic DNA. The sense oligonucleotide corresponded to positions 10021-10041 of the PRL- 1 gene and had the sequence (iii), while the antisense oligonucleotide corresponded to positions 100503-100481 of the PRL-1 gene which had the sequence (iv). Based on the sequences of human red and green cone pigment cDNAs, sense (v) and antisense (vi) primers corresponding to positions 156-176 and 443-423 of the red/green cone pigment cDNA respectively, were used to generate a 288 bp PCR product from monkey oligo(dT)-primed first strand cDNA. 3 mul of each unpurified PCR product was incubated with topoisomerase activated adaptors for 5 min, at room temperature. The modified acceptor DNA was amplified by PCR with primers specific to the target cDNA sequence. The purified PCR products with T7 promoter were sequenced using T7 or gene-specific primers. Sequencing confirmed the identity of the PCR products as T7 promoter-linked human PRL-1 gene and red/green cone pigment of cDNA sequences, respectively.
5'-TAATACGACTCACTATAGGGACCCTTGGTGCACCA-3' (i); 5'-AGGGTCCCTAT-3' (ii); GAAGCACATGTCTTTAATGTC (iii); GAACTAACATTAATACACATCAC (iv); GTACCACCTCACCAGTGTCT (v); and AAATGATGGCCAGAGACCA (vi).(19 pages)

L2 ANSWER 4 OF 19 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
ACCESSION NUMBER: 2002-04909 BIOTECHDS
TITLE: Amplifying gene-specific(GS) polynucleotide by producing population of DNA molecules having adapter molecule and anchor primer that has captureable group, synthesizing and amplifying single-stranded GS polynucleotide;
useful for research, infectious disease, Alzheimer disease, cancer, Duchenne muscular dystrophy diagnosis, DNA typing, paternity testing, forensics, crop improvement, animal breeding, occupational hazard screening and highthroughput DNA sequencing
AUTHOR: MULLER R; RIDDLE G H; GLASS J R
PATENT ASSIGNEE: DIGITAL GENE TECHNOLOGIES INC
PATENT INFO: WO 2001083696 8 Nov 2001
APPLICATION INFO: WO 2000-US13807 28 Apr 2000
PRIORITY INFO: US 2000-560845 28 Apr 2000
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2002-114150 [15]
AN 2002-04909 BIOTECHDS
AB DERWENT ABSTRACT:
NOVELTY - Amplifying (M1) a gene-specific (GS) polynucleotide by: (a) synthesizing double-stranded (ds) DNAs containing GS polynucleotide which has an anchor primer (II) with a captureable group (CG) at the 3' end; (b) ligating a ds adapter molecule to each dsDNA; (c) synthesizing a

single-stranded (ss) GS polynucleotide (I) using a GS oligonucleotide primer having a second CG; and (d) amplifying (I) after isolation.

DETAILED DESCRIPTION - Amplifying (M1) a GS polynucleotide comprises:(a) synthesizing a population of double-stranded dsDNA molecules containing a GS polynucleotide and having a 3' end and a 5' end, using (II) having a first captureable group (CG), where (II) is at the 3' end of each dsDNA molecule; (b) ligating a ds adapter molecule to the 5' end of each dsDNA molecule producing dsDNA molecules comprising both (II) and an adapter molecule; (c) synthesizing (I) using a GS oligonucleotide primer having a second CG, where the CG of the GS oligonucleotide primer is different from the CG of (II); (d) isolating the (I); and (e) amplifying the isolated GS polynucleotide using both a GS primer and primer that hybridizes to either a sequence located in the anchor primer or a sequence located in the adapter molecule. INDEPENDENT CLAIMS are also included for the following: (1) an isolated polynucleotide (anchor primer, II) (PN1) having an attached CG such as S1, S2, or comprises a sequence of 65 nucleotides (S45, S46), given in the specification; (2) an isolated ds polynucleotide (an adapter molecule) (PN2) comprising (S13) and the complementary sequence (S14); and (S15) and the complementary sequence (S16); (3) an isolated polynucleotide (adapter molecule amplification primer) (PN3) comprising (S17), (S18), and (S19); (4) an anchor amplification primer oligonucleotide (III) having an attached biotin group comprising (S20); (5) an anchor amplification primer oligonucleotide (IV) having an attached primary amine comprising (S21); (6) an anchor amplification primer oligonucleotide (V) comprising the (S23); (7) a composition comprising (V) and a carrier; (8) articles of manufacture comprising PN1 or PN2, and comprising a streptavidin-coated substrate or NOS-coated substrate, respectively; (9) a kit (VI) suitable for isolating and sequencing GS polynucleotide from a population of DNA or RNA polynucleotides comprising at least one ss anchor primer oligonucleotide having an attached CG and at least one ds adapter oligonucleotide, for at least one assay and suitable containers; (10) isolating (M2) a GS polynucleotide involving: (a) synthesizing a population of dsDNA molecules having a 3' end and a 5' end; (b) ligating a ds adapter molecule to the 5' end of each dsDNA molecule; (c) attaching at least one GS oligonucleotide primer having a CG to a solid substrate, where the GS primer is attached via the CG; (d) synthesizing (I) using the attached GS primer(s); (e) purifying (I); and (f) amplifying the GS polynucleotide(s) using a GS primer and a primer that hybridizes to a sequence located in the adapter molecule; (11) a recombinant construct into which is cloned a GS polynucleotide isolated according to (M2); and (12) a host cell comprising (11); and (13) an isolated polynucleotide (C-H).
5'-NAATTCAACTGGAAGCGGCCGCAGGAA(T)18-3' (S1) N = biotinylated guanidylate nucleotide; 5'-NAATTCAACTGGAAGCGGCCGCAGGAA(T)18-3' (S2) N = an aminated guanidylate nucleotide; 5'-ATAGCCTGCAGGTAATACGACTCACTATAGGGAC
TAGTCGACGGACCGCTAGCATCAGATC-3' (S13) 5'-GATCTGATGCTAGCGGTCCGTCGACTAGTCCC
TATAGTGAGTCGTATTACCTGCAGG-3' (S14) ACGAGCGGATAACAATTTCACACAGGGCGGCCGCTA
ATACGACTCACTATAGGGGTCGAC (S15) GTCGACCCCTATAGTGATTCGTATTAGCGGCCGCCCTGTG
TGAAATTGTTATCCGCTCGT (S16) CCTGCAGGTAATACGACTCACTATAGG (S17)
TAGTCGACGGACCGCTAGCATCAGATC (S18) CCGCTAATACGACTCACTATAGGGGTCGAC
(S19) 5'-NAATTCAACTGGAAGCGGCCGCAGGA-3' (S20, (S21) 5'-
NAATTCAACTGGAAGCGGCCGCAGGA-3' (S21) GAATTCAATGGAAGCGGCCGCAGGAAT (S23)
NAATTCAACTGGAAGCGGCCGCAGGAATTTTTTTTTTTTTTTTTT (C)
NAATTCAACTGGAACCTGCAGGAGGAATTTTTTTTTTTTTTTTTT (D)
NAATTCAACTGGAAACTAGTAGGAATTTTTTTTTTTTTTTTTT (E)
NAATTCAACTGGAAGTCGACAGGAATTTTTTTTTTTTTTTTTT (F)
NAATTCAACTGGAACGGATCCGAGGAATTTTTTTTTTTTTTTTTT (G)
NAATTCAACTGGAAGCTAGCAGGAATTTTTTTTTTTTTTTTTT (H) N = A, G, C, and T and is covalently bound to a CG such as biotin, primary amines, avidin, streptavidin, neutravidin, primary carboxylates, thiol alcohols, thiol carboxylates, carbonyls, sugars, lipids and peptides.

BIOTECHNOLOGY - Preferred Method: (M1) further comprises the additional step of sequencing the amplified, isolated GS polynucleotide.

(II) comprises the sequence of (A). The ds adapter molecules comprise the sequence of at least one rare restriction enzyme site, where the rare restriction enzyme site occurs in a mammalian genome less than of about one site per 15000 nucleotides. Preferably, the adapter molecule comprises the sequence of two or more rare restriction enzyme sites which are sequentially arranged, e.g., NotI, AscI, BaeI, FseI, SfiI, SgfI, SgrAI, SrfI, PacI, PmeI, PpuMI, RsrII, SapI, SexAI, Sse83871, SbfI, SpeI, SalI, RsrI, or NheI restriction enzyme sites. Most preferably, the double-stranded adapter molecule comprises the sequence of contiguous SbfI, SpeI, SalI, RsrI and NheI restriction enzyme sites. Also the ds **adapter** molecule comprises RNA **polymerase promoter** sequences such as T3, SP6 or T7 RNA **polymerase promoter** sequences. Preferably, the ds **adapter** molecule comprises the T7 **polymerase promoter** sequence. (M1) further comprises the step of amplifying the ds RNA molecules comprising both (II) and an adapter molecule before the step of synthesizing the single-stranded GS polynucleotide, where the dsDNA molecules are amplified using a 3' end primer that hybridizes to a sequence located in (II) and a 5' end primer that hybridizes to a sequence located in the adapter molecule. The 3' end amplification primer comprises (S20, S21) and the 5' amplification primer comprises (S17). Optionally, the 5' amplification primer comprises (S18). (M1) further comprises the additional step of synthesizing a population of single stranded RNA molecules from the population of dsDNA molecules comprising (II) and an adapter molecule, and digesting the dsDNA template molecules, before the step of synthesizing the (I). The GS primer employed in (M1) comprises the sequence (B). (M1) further comprises affixing: (a) the first CG of (II) of dsDNA molecule following synthesis of (I); or (b) a second CG of the GS primer of (I) following the synthesis of (I), where the first CG of (II), or the second CG of GS polynucleotide is affixed to a substrate comprising a coating such as streptavidin, avidin, neutravidin, N-oxysuccinimide ester, dimethyladipimidate-2-HCl and any one of the large number of compounds, given in the specification. Preferably, the first CG of (II) of dsDNA molecule or the GS primer of (I) is affixed to a substrate comprising a coating of streptavidin or N-oxysuccinimide ester. The purified (I) is amplified using a 5' end GS primer and 3' end amplification primer, where the 3' end amplification primer hybridizes to a portion of the sequence of (II). The 3' end amplification primer comprises (S23). Optionally, the purified (I) is amplified using a 3' end GS and a 5' end amplification primer, the 5' end amplification primer hybridizing to a portion of the anchor primer. The 5' end amplification primer comprises a sequence of (S17) or (S18). In (M2), the ds adapter molecule comprises the sequence of at least one rare restriction enzyme site occurring in a mammalian genome less than about one site per 15000 nucleotides. The ds **adapter** molecule comprises a T7 **polymerase promoter** sequence of (S13) or (S15). The GS primer comprises the sequence as described above. The purified (I) is amplified using a 5' end GS primer and a 3' end amplification primer that hybridizes to a sequence located in adapter molecule. The 3' end amplification primer preferably comprises (S17) and (S18). Optionally, (I) is amplified using a 5' end gene specific primer and 3' end amplification primer that hybridizes to a sequence located in an adapter molecule. Preferably, the 3' end amplification primer comprises a sequence of (S19). The method further involves cloning the isolated (I) into a vector to form a recombinant construct. Preferred Kit: The kit further comprises a ss amplification anchor primer oligonucleotide and ss amplification adapter primer oligonucleotide, a ss GS primer oligonucleotide having a CG, where the CG of the anchor primer oligonucleotide is different from the CG of the GS primer oligonucelotide. The GS primer oligonucleotide is attached to a substrate comprising a coating as described above. The substrate is preferably a multiwell plate. N'(N)a(T)b-3' (A) N' = a biotinylated or aminated nucleotide to which a CG is attached;a = an integer of 15-50; and b = an integer of 12-25. 5'-N'(N)c-3' (B) N' = a nucleotide to which a CG is

attached; c = an integer of 17-40; and N = a biotinylated or aminated nucleotide.

USE - (M1) is used for determining without cloning an extended sequence of a GS polynucleotide that has a partial gene sequence known by carrying out (M1) and then sequencing the amplified isolated GS polynucleotide without cloning (claimed). The method has several diagnostic and research applications, including genetic and infectious disease diagnosis, toxicology testing, individual identification, genetic research, occupational hazard screening and pharmaceutical development. The method can be used to detect mutations in target genes which may lead to disease conditions such as Alzheimer's disease, cancer, Duchenne muscular dystrophy, thus facilitating the diagnosis of genetic diseases as well as the identification of carriers of recessive genetic disorders. The method is used as a research tool in population studies aimed at determining which mutations within a target gene are associated with a disease and which mutations represent harmless polymorphisms. The method is used in the diagnosis and identification of infectious agents and for DNA typing of individuals in forensic and paternity testing. The method is used as a research tool for rapid widespread determination of new full-length DNA sequences and for the characterization of complex multigene disorders and other genetic traits. The method can be used for wide-scale sequencing of plant and animal genomes. The sequence data derived from the sequencing projects will aid in the development of pharmaceutical drugs under creation of improved crops and livestock.

ADVANTAGE - The method allows direct sequencing and identification of GS sequences and eliminates the process of cloning and screening clones, thus providing quicker sequence results. The capture of a GS first strand significantly reduces the background of non-specific polymerase chain reaction (PCR) products. Amplification of the entire adapted cDNA increases the ability to detect and sequence rare transcripts. The method is adaptable to multiwell formats, providing a high throughput system for generating extended and full-length sequences. The method is also adaptable to automation. The potential for automation allows simultaneous screening of large numbers of polymorphic markers in the individual.

EXAMPLE - Construction of a modified cDNA library (cDNAM), using a

0:140656

TITLE: Construction of small interfering RNA expression cassettes and expression libraries under control of a single RNA **polymerase** III **promoter** using a **polymerase** primer hairpin **linker**

INVENTOR(S): Li, Henry; Chatterton, Jon E.; Ke, Ning; Rhoades, Kristina L.; Wong-Staal, Flossie

PATENT ASSIGNEE(S): Immusol Incorporated, USA

SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2004009796	A2	20040129	WO 2003-US23239	20030723
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU				
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG				
US 2004115815	A1	20040617	US 2003-628587	20030723

PRIORITY APPLN. INFO.: US 2002-399040P P 20020724

AB The invention claims methods for construction of small interfering RNA (siRNA) expression cassettes using a polymerase primer hairpin linker. The expression cassette is constructed from a self-priming oligonucleotide comprising three segments (from 5' to 3' direction): (1) a 5' leader sequence between 4 and 27 nucleotides long with at least four consecutive adenylyl residues (complementary to the polIII transcription terminator) at its 3' end, (2) a coding sequence for the sense strand of an siRNA, preferably 11-27 nucleotides, and (3) a polymerase primer hairpin linker. The 5' leader sequence can include restriction site(s) for cloning siRNA coding sequences into expression cassettes. The polymerase primer hairpin linker forms a short stem-loop structure involving the 3' end of the self-priming oligonucleotide. The sequence encoding the corresponding antisense strand of the siRNA and the complement of the 5' leader sequence are produced by primer extension from the 3' end of the polymerase primer hairpin linker. The product of the primer extension reaction includes a stem-loop that must be denatured. Blocking primers are then annealed to the 5' and 3' ends of the denatured DNA. A complementary strand for the entire mol. is synthesized, thereby producing a duplex DNA that can be used to complete the construction of the expression cassette. The methods allow rapid construction of a single transcriptional unit encoding both strands of a hairpin siRNA, regardless of sequence. Expression cassettes of the invention contain an RNA polymerase III-dependent promoter and regulatory elements for inducible transcription of siRNAs. In addition, the invention includes libraries comprising the expression cassettes of the invention, including vectors for transforming cells, such as replication-deficient retroviral vectors. Methods of the invention and siRNA expression vectors may be useful for elucidation of gene function and identification of novel genes. Specifically, the present invention relates to methods and compns. for improved functional genomic screening, functional inactivation of specific essential or non-essential genes, and identification of genes that are modulated in response to specific stimuli or encode recognizable phenotypic traits. The examples of the invention

GS polynucleotide isolated according to (M2); and (12) a host cell comprising (11); and (13) an isolated polynucleotide (C-H).
5'-NAATTCAACTGGAAGCGGCCGCAGGAA(T)18-3' (S1) N = biotinylated guanidylate nucleotide; 5'-NAATTCAACTGGAAGCGGCCGCAGGAA(T)18-3' (S2) N = an aminated guanidylate nucleotide; 5'-ATAGCCTGCAGGTAATACGACTCACTATAGGGAC TAGTCGACGGACCGCTAGCATCAGATC-3' (S13) 5'-GATCTGATGCTAGCGGTCCGTCGACTAGTCCC TATAGTGAGTCGTATTACCTGCAGG-3' (S14) ACGAGCGGATAACAATTTCACACAGGGCGGCCGCTA ATACGACTCACTATAGGGGTCGAC (S15) GTCGACCCCTATAGTGATTCGTATTAGCGGCCGCCCTGTG TGAAATTGTTATCCGCTCGT (S16) CCTGCAGGTAATACGACTCACTATAGG (S17) TAGTCGACGGACCGCTAGCATCAGATC (S18) CCGCTAATACGACTCACTATAGGGGTCGAC (S19) 5'-NAATTCAACTGGAAGCGGCCGCAGGA-3' (S20, (S21) 5'-NAATTCAACTGGAAGCGGCCGCAGGA-3' (S21) GAATTCAATGGAAGCGGCCGCAGGAAT (S23)
NAATTCAACTGGAAGCGGCCGCAGGAATTTTTTTTTTTTTTTTT (C)
NAATTCAACTGGAACCTGCAGGAGGAATTTTTTTTTTTTTTTTT (D)
NAATTCAACTGGAAACTAGTAGGAATTTTTTTTTTTTTTTTT (E)
NAATTCAACTGGAAGTCGACAGGAATTTTTTTTTTTTTTTTT (F)
NAATTCAACTGGAACGGATCCGAGGAATTTTTTTTTTTTTTTTT (G)
NAATTCAACTGGAAGCTAGCAGGAATTTTTTTTTTTTTTTTT (H) N = A, G, C, and T and is covalently bound to a CG such as biotin, primary amines, avidin, streptavidin, neutravidin, primary carboxylates, thiol alcohols, thiol carboxylates, carbonyls, sugars, lipids and peptides.

BIOTECHNOLOGY - Preferred Method: (M1) further comprises the additional step of sequencing the amplified, isolated GS polynucleotide. (II) comprises the sequence of (A). The ds adapter molecules comprise the sequence of at least one rare restriction enzyme site, where the rare restriction enzyme site occurs in a mammalian genome less than of about one site per 15000 nucleotides. Preferably, the adapter molecule comprises the sequence of two or more rare restriction enzyme sites which are sequentially arranged, e.g., NotI, AscI, BaeI, FseI, SfiI, SgfI, SgrAI, SrfI, PacI, PmeI, PpuMI, RsrII, SapI, SexAI, Sse83871, SbfI, SpeI, SalI, RsrI, or NheI restriction enzyme sites. Most preferably, the double-stranded adapter molecule comprises the sequence of contiguous SbfI, SpeI, SalI, RsrI and NheI restriction enzyme sites. Also the ds **adapter** molecule comprises RNA **polymerase promoter** sequences such as T3, SP6 or T7 RNA **polymerase promoter** sequences. Preferably, the ds **adapter** molecule comprises the T7 **polymerase promoter** sequence. (M1) further comprises the step of amplifying the ds RNA molecules comprising both (II) and an adapter molecule before the step of synthesizing the single-stranded GS polynucleotide, where the dsDNA molecules are amplified using a 3' end primer that hybridizes to a sequence located in (II) and a 5' end primer that hybridizes to a sequence located in the adapter molecule. The 3' end amplification primer comprises (S20, S21) and the 5' amplification primer comprises (S17). Optionally, the 5' amplification primer comprises (S18). (M1) further comprises the additional step of synthesizing a population of single stranded RNA molecules from the population of dsDNA molecules comprising (II) and an adapter molecule, and digesting the dsDNA template molecules, before the step of synthesizing the (I). The GS primer employed in (M1) comprises the sequence (B). (M1) further comprises affixing: (a) the first CG of (II) of dsDNA molecule following synthesis of (I); or (b) a second CG of the GS primer of (I) following the synthesis of (I), where the first CG of (II), or the second CG of GS polynucleotide is affixed to a substrate comprising a coating such as streptavidin, avidin, neutravidin, N-oxysuccinimide ester, dimethyladipimidate-2-HCl and any one of the large number of compounds, given in the specification. Preferably, the first CG of (II) of dsDNA molecule or the GS primer of (I) is affixed to a substrate comprising a coating of streptavidin or N-oxysuccinimide ester. The purified (I) is amplified using a 5' end GS primer and 3' end amplification primer, where the 3' end amplification primer hybridizes to a portion of the sequence of (II). The 3' end amplification primer comprises (S23). Optionally, the purified (I) is amplified using a 3' end GS and a 5' end amplification primer, the 5' end amplification primer hybridizing to a portion of the anchor primer. The 5' end amplification

primer comprises a sequence of (S17) or (S18). In (M2), the ds adapter molecule comprises the sequence of at least one rare restriction enzyme site occurring in a mammalian genome less than about one site per 15000 nucleotides. The ds **adapter** molecule comprises a T7 **polymerase promoter** sequence of (S13) or (S15). The GS primer comprises the sequence as described above. The purified (I) is amplified using a 5' end GS primer and a 3' end amplification primer that hybridizes to a sequence located in adapter molecule. The 3' end amplification primer preferably comprises (S17) and (S18). Optionally, (I) is amplified using a 5' end gene specific primer and 3' end amplification primer that hybridizes to a sequence located in an adapter molecule. Preferably, the 3' end amplification primer comprises a sequence of (S19). The method further involves cloning the isolated (I) into a vector to form a recombinant construct. Preferred Kit: The kit further comprises a ss amplification anchor primer oligonucleotide and ss amplification adapter primer oligonucleotide, a ss GS primer oligonucleotide having a CG, where the CG of the anchor primer oligonucleotide is different from the CG of the GS primer oligonucelotide. The GS primer oligonucleotide is attached to a substrate comprising a coating as described above. The substrate is preferably a multiwell plate. N'(N)a(T)b-3'   (A) N' = a biotinylated or aminated nucleotide to which a CG is attached;a = an integer of 15-50; and b = an integer of 12-25. 5'-N'(N)c-3'   (B) N' = a nucleotide to which a CG is attached; c = an integer of 17-40; and N = a biotinylated or aminated nucleotide.

USE - (M1) is used for determining without cloning an extended sequence of a GS polynucleotide that has a partial gene sequence known by carrying out (M1) and then sequencing the amplified isolated GS polynucleotide without cloning (claimed). The method has several diagnostic and research applications, including genetic and infectious disease diagnosis, toxicology testing, individual identification, genetic research, occupational hazard screening and pharmaceutical development. The method can be used to detect mutations in target genes which may lead to disease conditions such as Alzheimer's disease, cancer, Duchenne muscular dystrophy, thus facilitating the diagnosis of genetic diseases as well as the identification of carriers of recessive genetic disorders. The method is used as a research tool in population studies aimed at determining which mutations within a target gene are associated with a disease and which mutations represent harmless polymorphisms. The method is used in the diagnosis and identification of infectious agents and for DNA typing of individuals in forensic and paternity testing. The method is used as a research tool for rapid widespread determination of new full-length DNA sequences and for the characterization of complex multigene disorders and other genetic traits. The method can be used for wide-scale sequencing of plant and animal genomes. The sequence data derived from the sequencing projects will aid in the development of pharmaceutical drugs under creation of improved crops and livestock.

ADVANTAGE - The method allows direct sequencing and identification of GS sequences and eliminates the process of cloning and screening clones, thus providing quicker sequence results. The capture of a GS first strand significantly reduces the background of non-specific polymerase chain reaction (PCR) products. Amplification of the entire adapted cDNA increases the ability to detect and sequence rare transcripts. The method is adaptable to multiwell formats, providing a high throughput system for generating extended and full-length sequences. The method is also adaptable to automation. The potential for automation allows simultaneous screening of large numbers of polymorphic markers in the individual.

EXAMPLE - Construction of a modified cDNA library (cDNAM), using a biotinylated anchor primer or an aminated anchor primer was carried out. To make a biotinylated library poly A+ selected mRNA was reacted with biotinylated anchor primer 5'-NAATTCAACTGGAAGCGGCCGCAGGAA(T)188-3' (where N is biotinylated guanidylate nucleotide) (S1) or a sequence of 65 nucleotides (S45), given in the specification. The resulting mRNA/anchor

primer mixture was incubated at 70 degrees Centigrade for 10 minutes and then quickly chilled on ice to stop the reaction. The first strand reaction mixture was prepared by combining the mRNA/anchor primer mixture with a standard cDNA producing mixture. The first strand reaction mixture was incubated and then the first strand synthesis was terminated. Second strand cDNA synthesis was then performed and obtained. Double-stranded cDNA was purified. To a modified cDNA library, such as a biotinylated or aminated cDNA library an adapter having a sequence of 5'-ATAGCCTGCAGGTAATACGACTCACTATAGGGACTAGTCGACGGACCGCTAGCATCAGATC-3' (S13) and the complementary sequence 5'-GATCTGATGCTAGCGGTCCGTCGACTAGTCCCTATAGTG AGTCGTATTACCTGCAGG-3' (S14) was annealed creating a double-stranded DNA molecule with one cohesive end (5' sense) and one blunt end (3' sense). The adapted modified cDNA library (acDNAM) was then produced. Amplification of an a cDNAM library using anchor amplification primers (An) and adapter amplification primers (Ap) was carried out to generate an amplified acDNAM (aacDNAM). The complete acDNAM was amplified using a pair of primers, one of which was located in the anchor sequence and one of which was located in the adapter sequence. An oligonucleotide of either CCTGCAGGTAATACGACTCACTATAGG (S17), or TAGTCGACGGACCGCTAGCATCAGATC (S18) was used to amplify an a cDNAM having an adapter molecule of (S13) and oligonucleotide of CCGCTAATACGACTCACTATAGGGGTCGAC (S19) was used to amplify an acDNAM having an adapter molecule of ACGAGCGGATAACAATTTCACACAGGGCGGCCGCTAATACGACTCACTATAGGGGTCGAC (S15). In addition, an oligonucleotide of either 5'-NAATTCAACTGGAAGCGGCCGCAGGA-3' (S20) or 5'-NAATTCAACTGGAAGCGGCCGCAGGA-3' (S21) was used to amplify an acDNAM having an anchor primer of (S1) or 5'-NAATTCAACTGGAAGCGGCCGCAGGAA(T)18-3' (S2) (where N is an aminated guanidylate nucleotide). Generation of an aminated gene-specific first strand cDNA using an acDNAB or aacDNAB template and an aminated gene-specific primer was then carried out. The single-stranded gene-specific cDNA was synthesized in either the sense and antisense orientation depending on the orientation of the gene-specific primer. A gene specific cDNA synthesized from a gene-specific primer having sense orientation contained the anchor sequence. A gene-specific cDNA synthesized from a gene-specific primer having antisense orientation contained the adapter sequence. Alternately, the generation of a biotinylated gene-specific first strand cDNA using an acDNAA or aacDNAA template and a biotinylated gene-specific primer was carried out. The aminated gene-specific cDNA was isolated from a complete acDNAB library and aminated the gene-specific single strand cDNA using two independent purification steps. The first step involved the association of the biotinylated adapted cDNA library to streptavidin- and oligodT-coated Dynabeads (RTM) and physical separation of the acDNAB library through magnetic forces from the aminated gene-specific single stranded cDNA. The second step involved the attachment of the aminated gene-specific cDNA to NOS-coated polymerase chain reaction (PCR) plates and elimination of the remaining acDNAB library molecules through a stringent washing procedure. The biotinylated gene-specific cDNA was isolated from the reaction products by using two independent purification steps. The first step involved the attachment of the aminated acDNAA library to NOS-coated plates and recovery of the biotinylated gene-specific cDNA in solution. The second step involved the association of the biotinylated gene-specific cDNA to streptavidin coated Dynabeads (RTM) and physical separation from the contaminating aminated acDNAA through magnetic forces. A purified gene-specific first strand cDNA was prepared and the gene specific first strand cDNA directed in the antisense orientation was amplified using an antisense gene-specific primer and a sense adapter sequence primer (i.e., (S17), (S18), (S19), or ACGAGCGGATAACAATTTCACACAGG (S44)). An attached gene-specific first-strand cDNA directed in the sense orientation was amplified using a sense gene specific primer and an antisense anchor sequence primer (i.e., GAATTCAATGGAAGCGGCCGCAGGAAT (S23) or AATTATTAACCCTCACTAAAGGGACAACTGGAAGCGGCC (S49)). A purified, amplified gene-specific PCR product was cycle sequenced. (115 pages)

Production of sequence-tagged polynucleotides;
sequence tag oligonucleotide production

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AB A new method for producing a sequence tag from a polynucleotide involves: digesting the polynucleotide with a type II restriction endonuclease to produce a 1st cohesive end; providing a **promoter-linker** cassette comprising an RNA-**polymerase promoter**, a restriction site for a type IIs restriction enzyme and a 2nd cohesive end complementary to the 1st cohesive end produced by the type II restriction endonuclease; ligating the digested polynucleotide to the cassette by joining the 1st cohesive end and the 2nd cohesive end; digesting the ligated product with the type IIs restriction enzyme; and transcribing the resulting construct from the promoter, thereby producing a sequence tagged polyribonucleotide. Also claimed are: a method for analyzing a population of polynucleotides for the presence of a known sequence, which involves producing sequence tags from the polynucleotides, combining the tags with oligonucleotide(s) derived from the known sequence, and determining whether the tags hybridize to 1 or more oligonucleotides; a promoter-linker cassette. (9pp)

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Detailed Description Text - DETX (58):

In one aspect of the invention, telomerase extended products are replicated by means of the action of an RNA polymerase. A variety of methods for replicating nucleic acids using an RNA polymerase are known, e.g., nucleic acid sequence-based amplification (Compton, 1991, Nature 350:91-92), self-sustained sequence replication (Guatelli et al., 1990, Proc. Natl. Acad. Sci. USA 87:1874-1878), and strand displacement amplification (Walker et al, 1992, Proc. Natl. Acad. Sci. USA 89:392-396). In a preferred embodiment, an RNA polymerase that utilizes a single stranded DNA template (e.g., N4 RNA polymerase) is employed to synthesize an RNA copy from a single-stranded DNA template using a promoter sequence at the 5' end of the template. The telomerase substrate can comprise a promoter sequence operably linked to its 5' end or the promoter can be ligated to the substrate after extension of the substrate and prior to replication with the RNA polymerase. Many RNA transcripts can be generated from a single DNA template thereby increasing the number of the target molecules. One can optionally employ a reverse transcriptase to make DNA copies of the RNA transcript.

Detailed Description Text - DETX (60):

As noted above, amplification can also be achieved by using nucleic acid sequence-based amplification (NASBA). In this embodiment, an RNA polymerase (such as T7 RNA polymerase) synthesizes RNA copies of the extended telomerase substrate essentially as described above. A reverse transcriptase is used to synthesize DNA copies of the RNA, RNaseH degrades the RNA strand and the single stranded DNA acts as a template for RNA synthesis, thus providing cyclic amplification. Alternatively, reverse transcriptase can be used to synthesize cDNAs, or RNA polymerase can be used to extend primers, and the products can be

amplified by primer extension (e.g., with PCR). These and other variations of the present method will be apparent to those of skill in the art upon consideration of this disclosure. In these embodiments, the presence and level of telomerase activity is correlated to the presence of RNA copies of the extended telomerase substrate. As in other embodiments, a wide variety of primers can be designed (for example, to decrease background), as would be apparent to one of ordinary skill in the art.

mbining the technique of RNA fingerprinting and **differential display** to obtain **differentially expressed** **mRNA**

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CORPORATE SOURCE: CLONTECH Laboratories, Inc., Palo Alto, CA, 94303-4230, USA
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AB We have modified recently developed methods of RNA fingerprinting and **differential display** based on arbitrarily primed PCR which can be used for detection and cloning of differentially expressed mRNAs. Our protocol requires only a single cDNA synthesis for each different RNA sample, in contrast to the multiple cDNA reactions required for **differential display** method, followed by **selective amplification** of cDNA **sequence** fraction by arbitrary and **oligo(dT)** primers. We have shown that the longer primers (25-29-mers) allow the use of optimal dNTP concentration and higher stringency PCR. Further improvements include using TaqStart antibody for hot start PCR and thermostable enzyme mixes suitable for long-distance PCR. Long-distance PCR enables the method to display bands of up to 2 kb and should result in a higher fidelity of PCR products to the original RNA template. When these improvements are combined the resulting method is highly reproducible, and more than 85% of the differentially expressed bands can be confirmed by Northern blot anal.

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A Novel Strategy for Identifying Differential Gene Expression: An Improved Method of **Differential Display** Analysis

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AB We propose a novel alternative approach, an advanced method for recently developed strategies, for identifying differentially expressed genes. Firstly, double-stranded cDNAs were digested using Sau3AI and the 3'-end restriction fragments of the cDNA were ligated to a double-stranded adapter. Next, the restriction fragments were directly amplified using several combinations of adapter-specific primers and FITC-labeled oligo dT primers. The selected cDNA fragments were displayed on a polyacrylamide gel. Neither nested PCR nor purification of 3'-end fragments are necessary. We examined the validity of this approach by evaluating gene expression changes during granulocytic differentiation of HL-60 cells. This method can theor. detect almost all gene expression changes more rapidly and through simpler manipulations than by any other approach. (c) 1999 Academic Press.